

# CLAIMS

What is claimed is:

[c1]

1. (currently amended) A method that is useful for quantitatively changing deamidation rates of Asn and Gln residues in peptides and proteins or in other molecules that contain these amides, including hormones and drugs and modifications of peptides, proteins, hormones and drugs.

(a) This can be done through substitution of the *carboxyl-side* residue to the amide with another residue, using the experimentally determined values in Tables 1 and 2.

(b) For example, suppose an investigator is confronted with the sequence -IleAsnAla- in a particular protein. From Table 1, he knows that the deamidation rate is 25.9 days under the conditions in the table. If a deamidation rate of, for example, 150 days is more desirable, an examination of the table shows that this can be done by changing the sequence to -IleAsnLeu-, which has a half-time of 154 days.

(c) Table 1 is to be used for Asn sequences and Table 2 for Gln sequences.

~~The invention of a technique for changing deamidation rates of Asn and Gln residues in peptides, hormones, proteins and peptide-like, hormone-like, and protein-like molecules through modification of the residue or residue-like structure to the right (or carboxyl side) of the amide.~~

~~(a) This can be done through substitution of either a natural or non-natural amino acid side chain.~~

~~(b) The effects of many such modifications are known based on experimental work which has been carried out.~~

~~(c) From the results described in (b) many other similar modifications can be inferred.~~

[c2]

2. (currently amended) A method that is useful for quantitatively changing deamidation rates of Asn and Gln residues in peptides and proteins or in other molecules that contain these amides, including hormones and drugs and modifications of peptides, proteins, hormones and drugs.

(a) This can be done through substitution of the *amino-side* residue to the amide with another residue, using the experimentally determined values in Tables 1 and 2.

(b) For example, suppose an investigator is confronted with the sequence -IleAsnLeu- in a particular protein. From Table 1, he knows that the deamidation rate is 154 days under the conditions in the table. If a deamidation rate of, for example, 100 days is more desirable, an examination of the table shows that this can be done by changing the sequence to -GlyAsnLeu-, which Table 1 shows, has a half-time of 104 days.

(c) Table 1 is to be used for Asn sequences and Table 2 for Gln sequences.

~~The invention of a technique as in claim 1, but modifying the residue or residue-like structure to the left (or amino side) instead of to the right.~~

[c3]

3. (currently amended) A method that is useful for quantitatively changing deamidation rates of amides residues in peptides and proteins or in other molecules that contain these amides, including hormones and drugs and modifications of peptides, proteins, hormones and drugs through the swapping of Asn for Gln or of Gln for Asn.

(a) This is quantitatively done by reference to both Tables 1 and 2.

(b) For example, suppose an investigator is designing a drug for which a part of the structure contains the sequence -LeuAsnGly-. He can tell from Table 1 that the half-time is likely to be about 1 day (unless it is suppressed by three-dimensional structure) and wishes to slow deamidation down to improve the drugs shelf-life. Table 2 shows that this can be done simply by substituting the sequence -LeuGlnGly- which now has a half-time of 670 days.

~~The invention of a technique for changing deamidation rates of Asn and Gln residues in peptides, hormones, proteins and peptide-like, hormone-like, and protein-like molecules by swapping Gln for Asn or Asn for Gln.~~

[c4]

4. (currently amended) A method that is useful for quantitatively changing deamidation rates of Asn and Gln residues in peptides and proteins or in other molecules that contain these amides, including hormones and drugs and modifications of peptides, proteins, hormones and drugs, through modification of molecular structures nearby the amide in space, but not part of the residues immediately to the carboxyl side or amino side of the amide, as determined by a three-dimensional prediction procedure.

(a) This procedure identifies certain structural components that can have an effect on the deamidation rate as set out in paragraphs [0034] to [0057].

(b) For example, the beta-Lys-Asn145-His sequence of hemoglobin is not in an alpha-helix or in a loop between two beta sheets, so S1 through S4=0, S5=1. There is one hydrogen bond to the amide side chain nitrogen and one other to be broken to form the imide, but there are none to the amide carboxyl or the backbone nitrogen, so S6=0, S7=1, S8=0, and S9=1. This Asn is near the carboxyl end of the chain and one residue from an alpha-helix on the amino side, so S10=0, S11=1, and S12=5. From Table 1, the primary sequence half-time is 1-.5 days. Therefore  $CD=(0.01)(10.5)e^{(0.48)[(1)(1) + (2)(1)+(2)(1-0)+(0.2)(4)]}=(0.105)e^{(0.48)(5.8)} = (0.105)(16.184)=1.70$ . The predicted half-time is (100)CD so the half-time is estimated to be 170 days.

If an investigator wishes to change this half-time to, for example, around 100 days, he can see from the parameters used to predict the rate that one possibility is to eliminate the amide side chain nitrogen hydrogen bond. Thus S7=0 and he can calculate  $CD=(0.01)(10.5)e^{(0.48)[(2)(1)+(2)(1-0)+(0.2)(4)]}=(0.105)e^{(0.48)(4.8)} = (0.105)(10.014)=1.05$ . The predicted half-time is now (100)CD so the estimated half-time is now 105 days.

~~The invention of a technique for changing deamidation rates of Asn and Gln residues in peptides, hormones, proteins and peptide-like, hormone-like, and protein-like molecules through modification of structures in the near vicinity to the amide as determined by a three-dimensional prediction procedure.~~

[c5]

5. (currently amended) A method that is useful for quantitative modification of the rate of isomerization, for Asn, and Gln residues using the technique outlined in paragraphs [0034] to [0057].

(a) For example, it is known that isomerization and deamidation occur through the same mechanisms. Thus the isomerization of Asn or Gln cannot take place without deamidation occurring. If it is found that 1/2 of deamidation for a particular peptide is accompanied by isomerization, then an investigator would know that the deamidation of, for example, the sequence -AlaAsnAla- in a drug with no three-dimensional interference, would have a primary half-time (using Table 1) of 22.5 days and that the isomerization half-time would be 45 days. If it is desirable to change this rate to about 75 days, he can do this by redesigning the drug to include a hydrogen bond in the S7 position and the isomerization rate would now be  $(45)e^{(0.48)(1)}=73$  days.

~~The extension of the inventions in claims 1, 2, 3, and 4 to isomerization, chain cleavage, or other reactions similar to deamidation for Asn and Gln residues or residue-like structures in a similar manner to those described in claims 1, 2, 3, and 4.~~

[c6]

6. (currently amended) A method that is useful for quantitative modification of the rate of isomerization, for Asp, and Glu residues using the technique outlined in paragraphs [0034] to [0057].

(a) For example, the rates of isomerization for Glu and Asp are about 100 fold slower than for deamidation. If one wishes to know the rate of isomerization of, for example, a peptide containing the sequence -GlyAspAla-, reference to Table 1 shows that the half-time for deamidation of this sequence is 21.1 days. If there are no three-dimensional constraints the method reduces to this half-time multiplied by 100 for isomerization. Thus the isomerization half-time can be estimated as  $(21.1)(100) = 2100$  days. In order to slow this reaction down, an investigator could add a hydrogen bond in the C<sub>59</sub> category. This would slow it down by a factor of  $e^{(0.48)(2)}=2.6$ , so the new half-time would be  $(2.6)(2100) = 5460$  days.

~~The extension of claims 1, 2, 3, and 4 to the isomerization, chain cleavage or other reactions similar to deamidation for Asp and Glu side chains.~~

[c7]

7. (currently amended) A method that is useful for quantitative modification of the rate of chain cleavage, for Asn, Gln, Asp, and Glu residues using the technique outlined in paragraphs [0034] to [0057].

(a) For example, if a particular sequence in a protein, for example, -LeuAsnPro- is found to undergo cleavage with a half-time of 200 days, and an investigator wishes to speed this reaction up to around 100 days, he may be able to do so by removing a hydrogen bond. If C<sub>59</sub> is still 2.0 and one hydrogen bond exists in this position, removing it would speed the reaction up by a factor of  $e^{(0.48)(2)}=2.6$  and the new half-time is 77 days.

~~The use of the inventions in claims 1, 2, 3, 4, 5, and 6 to predict the rates of deamidation, isomerization, chain cleavage or similar related reactions.~~

## ABSTRACT OF THE DISCLOSURE